Nanowires and Living Cells

Injection of Quantum Dots and Genes Demonstrated

Two Materials Sciences Division scientists, with support from the MSD Molecular Foundry have achieved breakthroughs in integrating nanowires with living cells. First, Alex Zettl in collaboration with Carolyn Bertozzi in the Foundry Biological Nanostructures Facility, used multiwalled carbon nanotube nanoinjectors to deliver quantum dots into cells. Then, Peidong Yang and his postdoctoral fellow Kim Woong, collaborating with Bruce Conklin of the Gladstone Institute of Cardiovascular Disease, University of California at San Francisco, integrated silicon nanowires with embryonic stem cells.

Nanotechnology is of increasing interest to the biological sciences. For example, the use of nanoparticles in optical and magnetic resonance imaging is well-established and nano-scale probes for detecting or interrogating individual cells are being developed. However, more refined methods are required to directly and selectively introduce molecules and particles into the cell through nanotechnology.

In one demonstration of this promise, Bertozzi in the Foundry Biological Nanostructures Facility, in collaboration with Zettl attached multiwall carbon nanotubes (MWNTs) to an atomic force microscope (AFM) tip. They attached optically active "quantum dots" to the MWNTs by a linker molecule with a pyrene moiety at one end, a biotin moiety at the other end, and a disulfide bond in the middle. The nonpolar pyrene binds to the nonpolar nanotube, while the biotin binds to a streptavidin moiety on the surface of the quantum dot, thus converting the tubes into quantum dot carriers. The nano-manipulation capabilities of the AFM were used to locate individual cells and use the MWNT "nanoneedle" to penetrate the cell membrane. The reducing environment inside the cell cleaved the disulfide bond, releasing the quantum dot. Subsequent optical measurements confirmed that the technique can deliver a discrete number of molecules to the cell's interior. There was no discernible membrane or cell damage.

In a second demonstration of the application of nanotechnology, Yang and Kim of the Foundry Inorganic Nanostructures Facility with Foundry user Conklin, grew mouse embryonic stem (mES) cells on a Si surface supporting a vertically aligned array of silicon nanowires. The nanowires penetrated individual cells spontaneously during cell growth. In a preliminary demonstration of gene delivery, DNA was electrostatically deposited on an array of wires. Cells incubated on this array showed evidence, via differentiation, of successful gene delivery, although the yield for this was less than 1%.

This work shows the promise of integrating nanowires and tubes with living cells, and is an example of the interdisciplinary work that is the hallmark of LBNL's Molecular Foundry. There are a number of promising directions for the new techniques. Using the unique capabilities of the nanoinjector, other biomolecules such as DNA and RNA, or synthetic structures such as polymers, dendrimers and nanoparticles can be delivered into cells. The use of AFM positioning will also allow delivery of cargo to specific subcellular compartments. In addition, cells such as bacteria that are too small for microinjection, (the currently used technique which involves tiny glass tubes), should be amenable to nanoinjection The Si nanowire array also holds great promise for inducing embryonic stem cells to become specific, differentiated tissue cells. This would be a far simpler technique than the current complex *in vivo*, process, involving the control of a cell's genetic machinery with signals from its surrounding environment. In addition to the gene delivery method discussed here, cell differentiation into specific tissue types could be induced through electrical pulses or chemicals transmitted via nanowires.

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Xing Chen, Andras Kis, A. Zettl, and Carolyn R. Bertozzi, "A cell nanoinjector based on carbon nanotubes," Proc. Nat. Acad. Sci104, 8218 (2007)

Woong Kim, Jennifer K. Ng, Miki E. Kunitake, Bruce R. Conklin, and Peidong Yang, "Interfacing Silicon Nanowires with Mammalian Cells," JAmerChemSoc29, 7228 -7229 (2007)

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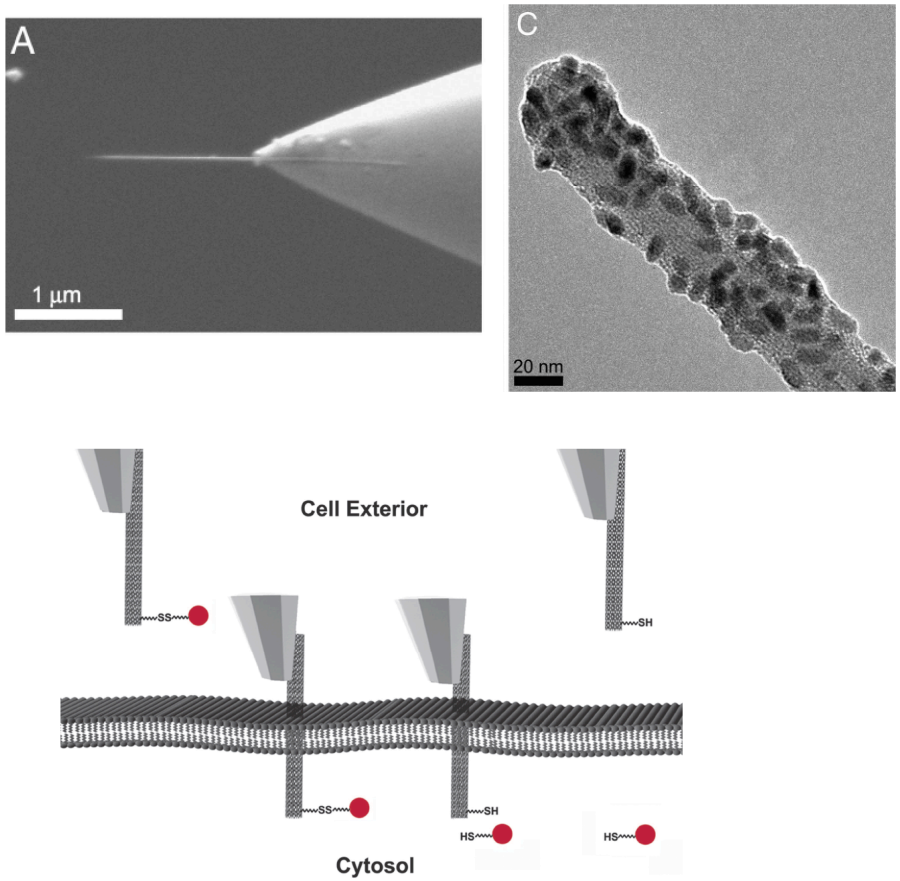
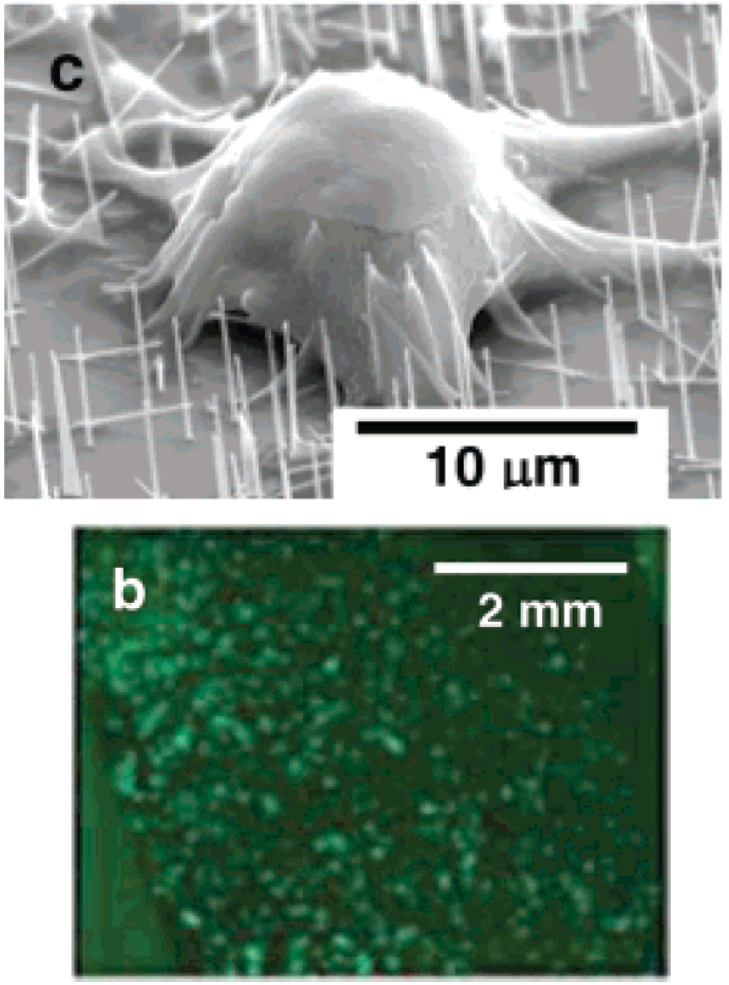


Injection of quantum dots (QD). *Multiwalled carbon nanotube (MWNT) on an AFM tip (top left) is coated with quantum dots (right). Schematic (bottom) shows the nanoneedle with attached QD cargo penetrating a cell membrane. After reductive splitting of the S-S bond, the cargo is released and the nanoneedle is retracted.*



Mouse embryonic stem cells *penetrated with Si nanowires (top). The fluorescence microscopy image (bottom) demonstrates the apparently normal, healthy proliferation of penetrated cells.*